REMARKS

Reconsideration is requested.

Claims 1-18 and 21-39 are pending. Claims 9-18, 21-32 and 35-39 have been withdrawn from consideration. Claims 1-8, 33 and 34 are under active consideration.

Claims 7 and 33 have been canceled, without prejudice, and claim 40 added above. No new matter has been added. Upon entry of the present Amendment, claims 1-6, 8-18, 21-32 and 34-40 will be pending. Claim 40 is believed to read on the elected subject matter.

The claims have been amended, without prejudice, to at least place the application in better condition for appeal. Entry of the present Amendment is requested. Support for the amendments may be found throughout the specification, including the originally-filed claims. The applicants note in this regard that the recitation of *Lactobacillus* in claim 1 above is supported by, for example, originally-filed claim 2. The recited size range of from 40 to 70 kd finds support, for example, at page 5, final paragraph. The recitation of highly basic, with a pI of at least 9, finds support, for example, at page 2, first paragraph of the specification. The reference to insertion sites finds support, for example, at page 7, lines 3 to 9, page 7, lines 23 and 24 and in pages 44 and 45 of the originally-filed specification. The revisions of claim 4 find support, for example, at page 43, second full paragraph of the specification. Newly added claim 40 finds support, for example, in claim 1 and also at page 5, last paragraph of the specification. Reference to residues 290 to 410 in claim 40 finds support, for example, at page 7, lines 20 to 23 of the specification. No new matter has been added.

The Examiner is requested to return an initialed copy of the PTO 1449 Form filed November 15, 2007, pursuant to MPEP § 609. A copy of same has not been received and a copy of same is not contained in the PTO IFW. Correction of the record in this regard is requested.

A completely initialed copy of the PTO 1449 Form indexed on June 15, 2007 in the PTO IFW which confirms the Examiner's consideration of WO98/33386A is requested, pursuant to MPEP § 609. While the Examiner has executed the entire form listing WO98/33386A on June 7, 2007, the Examiner has not included her initials in the left-hand column opposite WO98/33386A, as is believed to be required by MPEP § 609.

To the extent not obviated by the above amendments, the Section 112, first paragraph "enablement", rejection of claims 1-8, 33 and 34 is traversed. Reconsideration and withdrawal of the rejection are requested in view of the above and the following further comments.

Claim 1, as revised above, refers to the unmodified protein being from *Lactobacillus*. Moreover, the claim specifies a size range of from 40 to 70 kd, that the protein is highly basic with a pI of at least 9, as well as particular regions where insertion has been shown not to eliminate ability to crystallize. The claimed invention has been exemplified in the Examples of the present application.

As further described below, one of ordinary skill in the art will appreciate that the *Lactobacillus* surface layer proteins are a tightly related group of proteins which would be expected to behave in a similar behavior. The applicants submit that one of ordinary skill in the art will be able to make and use the claimed invention from the

description of the specification and such generally advanced knowledge of skill in the art. The combination of the guidance provided by the specification, the specific modified proteins described in the Example of the present application and what was known about how *Lactobacillus* surface layer proteins form a tightly related group lead to a conclusion that one of ordinary skill will be able to make and use the claimed invention without undue experimentation. Consideration of the following in response to the Examiner's comments regarding In re Wands 8 USPQ2d 1400, 1404 (Fed. Cir. 1988). See pages 6-9 of the Office Action of February 26, 2008.

Initially, the applicants note that the Wands panel reversed the Board of Appeals "enablement" rejection of the claimed methods finding that the Wands specification taught one of ordinary skill in the art how to make and use antibodies needed to practice the claimed method even though production of monoclonal antibodies necessary to practice the invention first required production and screening of numerous antibody producing cells or "hybridomas," since practitioners of art are prepared to screen negative hybridomas in order to find those that produce desired antibodies. The Wands panel found in this regard that those of ordinary skill in the art did not consider one "experiment" to be simply screening of one hybridoma but rather an entire attempt to make the desired antibody. Since the Wands record indicated that the amount of effort needed to obtain desired antibodies is not excessive and in view of applicants' demonstrated success the court held that the specification provided an enabling disclosure.

(i) *Nature of the invention*

The claims define a modified bacterial surface layer protein containing an internal insertion of a heterologous polypeptide, wherein the modified protein is able to crystallize.

The ability to crystallize, as specified by the claims, refers to the ability of the monomeric modified S proteins to spontaneously form a two-dimensional crystalline monolayer, that, under natural circumstances, comprises the surface layer that envelopes the entire bacterial cell.

The ability to crystallize, as specified in the claims, will not be confused by one of ordinary skill in the art with formation of the type of crystals used in X-ray crystallography to determine the three-dimensional structure of a protein and the difficulties which may be associated with such crystallisation. The applicants believe that the a general perception of the difficulty of preparing crystals for X-ray crystallography may have been artificially imported into the consideration of the current invention, where it is not relevant.

The Examiner's attention is drawn to Item 6 of the Declaration of Professor Pouwels previously filed that states that:

> "*Thus, reference to a crystalline structure in the present instance* ***does not refer to the formation of the type of crystal that is used to determine the three-dimensional structure of a protein in X-ray crystallography*** *and the difficulties of generating such three dimensional crystals that is commonly associated with X-ray crystallography.*" [emphasis added]

Thus, in considering whether or not the enablement requirements are met, the difficulty in producing crystals for X-ray crystallography is of no relevance and should not be taken into account.

(ii) *Breadth of the claims*

Claim 1 refers to the unmodified S protein originating from a *Lactobacillus* bacterium. The claims are based on one type of bacteria and the exemplification of the present application.

As set out previously in the Declaration from Professor Pouwels, *Lactobacillus* surface layer proteins represent a tight group of closely related proteins. The Examiner is requested to consider the following in further support of same.

The following Figure 1 provides a sequence alignment between the sequence of surface layer proteins from four different representative *Lactobacillus* bacteria. The Figure shows that there is more than 20% amino acid identity and over 60% amino acid similarity between the proteins. The different proteins are therefore clearly closely related.

Figure 1 - Alignment of S-layer proteins from different species of *Lactobacillus* showing sequence identity and similarity

Alignment data :
Consensus:
Residues conserved for 90 % or more (upper-case letters) : 99 is 22.45 %
Residues conserved for 40 % and less than 90 % (lower-case letters) : 288 is 65.31 %
Residues conserved less than 40 % (white space) : 33 is 7.48 %
Sequence 0001 : L.gallinarum AY597260
Sequence 0002 : L.acidophilus X71412
Sequence 0003 : L.helveticus X91199
Sequence 0004 : L.crispatus AJ007839

Secondary structure is indicated as H, helix; E, beta strand and C, coil

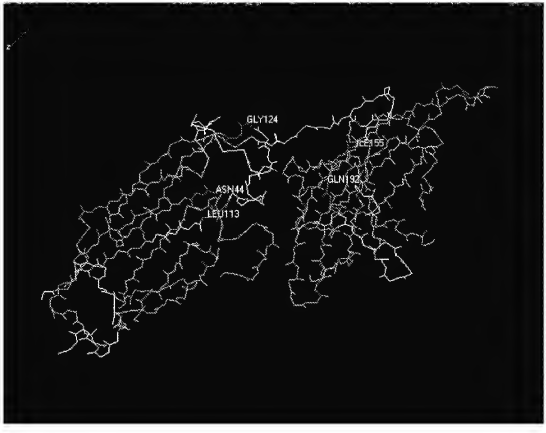
The Examiner is urged to appreciate that page 43, second full paragraph of the specification, describes the high level of sequence identity between different *Lactobacillus* S proteins, particularly in the C terminal third, but also in the remainder of the protein. One of ordinary skill in the art will appreciate that these proteins form a tightly related group which one of ordinary skill in the art will reasonably predict to behave in a similar manner.

In further support, the applicants provide the following Figure 2 which is a model of the four representative S layer proteins superimposed on each other showing that the proteins can be perfectly superimposed and hence have highly similar tertiary structure.

Figure 2

Superposition of S-proteins from different Lactobacillus S proteins shows their close structural similarity

Determination of the structure of the *S.epidermidis* adhesin SdrG has allowed protein modelling of *Lactobacillus* s-proteins. The results show that s-proteins from representative species (*L. acidophilus, L. helveticus, L. crispatus* and *L. gallinarum*) can be perfectly superpositioned, indicating that their overall tertiary structure is highly similar.



Green: *L. acidophilus*
Blue: *L. crispatus*
Pink (not visible): *L. helveticus*
Red: sites of insertions from Examples
White: regions of low or no secondary structure that are amenable to modification

Where the sequences overlap, only the *L. acidophilus* sequence (green) is visible.

Further, the following Figure 3 shows a comparison of the secondary structure of representative examples of *Lactobacillus* S proteins from four different species and shows that the four have an almost identical secondary structure. Figure 3 provides further persuasive evidence that one of ordinary skill in the art will find the evidence of the present specification to be reasonably predictive of the genus of the claimed invention.

Figure 3

Consensus secondary structure of the N-terminal part (SAN) of S-layer proteins from different species of *Lactobacillus*

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                  10        20        30        40        50        60        70        80        90       100       110
                   :         :         :         :         :         :         :         :         :         :         :
L.acidophilus     ATTINASSSAINTNTNAKIDVDVTPSVSAVAANTAN---NT-PAIAGNLTGTISASYNGKTYTANLKADTENATI---TAAGSTTAVKPAELA---AQVAYTV
SecStrCons        CCHHHCCCCCCCCCCCCCCEEEECCCCEEEEEECCCC   CC CEEEEEEEEEEEEECCCCEEEEECCCCCCCCEEE   EECCCCEEECCCCEE   ECCEEEE

                  120       130       140       150       160       170       180       190       200       210       220
                   :         :         :         :         :         :         :         :         :         :         :
Lact    TVSDVSFNFGSENAGFTVTLGSANSNVAFTGTNSDNQTETNVSTLKVKLQQNGVASLT-NVSIANVYAINTTDNSNVNFYQVTSGATVTNGAVSVNAD-NQSQVNVANVV
SecStrCons  EEECEEEECCCCCCCCCEEEEEECCCCCEEECCCCCCCCCCCCCCEEEEEECCCCEECCC EEEEEEEEEEEECCCCCCEEEEEEEECCCCCEECCCEEEEC CCCCCCHHHHH

                   230       240       250       260       270       280       290       300       310
                    :         :         :         :         :         :         :         :         :
Lact    AAI--NSKYEAAQYADKKLNTKTANTEDAIKA---ALFDQKLDVNSVGYFKAPHTFTVNVKSTGNTNGKSSTLPVVVTVPNVAEYT---VASVSFKI
SecStrCons  HHH  HCCCEEEEECCCCCCCCCCCHHHHHHH   HHHHCCCCCCCCEEECCCEEEEEEEEECCCCCCCEEEEEEEEECCCCCCC   CCCCCCC
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Alignment data :

Secondary structure is indicated as H, helix; E, beta strand and C, coil

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The above three Figures further demonstrate the relatedness of a group of *Lactobacillus* surface proteins and is submitted as persuasive evidence in support of the abilities of one of ordinary skill in the art to make and use the claimed invention from the teachings of the specification and the generally advanced level of skill in the art.

In further support, the Examiner is requested to reconsider Smit *et al* ((2001) *Journal of Molecular Biology* 305:245-257) and Boot *et al* ((1996) *Microbiology* 142: 2375-2384), both of record, which provided sequence alignment data and secondary structure data showing the similarity between *Lactobacillus* S proteins. The references further provide evidence that one region in a particular S protein from Lactobacillus would behave in an equivalent fashion to another. Such information would have confirmed for the ordinarily skilled person that the regions indicated to be effective for insertion would be effective in all *Lactobacillus* S proteins. The secondary structure information would also make it reasonably predictable that extrapolation from the specific insertion sites to the regions specified by the claims is reasonable.

As stated previously by Professor Pouwels in Item 9 of his Declaration:

> *"The term S protein therefore defines a narrow subset of proteins with one S protein typically being found for each bacterium.* ***The term does not refer to any and all proteins found in the bacterial cell membrane or bacterial cell wall****. The present claims are not therefore directed to hugely broad array of proteins. They are directed to a* ***very specific, tightly related, protein type, fulfilling the same role in each bacterium****. That is particularly the case now the claims have been amended to refer to the*

> *protein coming from a Lactobacillus bacterium"* [emphasis added]

The Examiner will appreciate that the claims refer to the unmodified protein having a weight of from 40 to 70 kd and being highly basic with a pI of at least 9, as well as insertions in one of the following regions: at a position from amino acids 1 to 20; at a position from amino acids 35 to 55; at a position from amino acids 100 to 130; at a position from amino acids 110 to 140; at a position from amino acids 193; and/or at a position from amino acids 340 to 360.

The Examiner's attention is further drawn to the Examples and to pages 44 and 45 of the specification. Table 2 shows that insertions within the regions specified by claim 1 result in retention of the ability to crystallize. The sites where insertion is shown to result in loss of ability to crystallize are not encompassed by the specified regions and hence the claims find support in the exemplifications of the specification.

The claims are submitted to be supported by an enabling disclosure.

(iii) *Direction or guidance presented in the specification*

The application provides substantive evidence that the claimed products are capable of crystallizing. In particular, for example, claim 1 recites that the unmodified protein is from *Lactobacillus* bacterium and also specifies that the insertion is in one of the specific regions demonstrated in the Example to not prevent crystallization.

As described on pages 44 and 45, and shown in the Table on page 45, of the specification, for example, modification within the specific regions of claim 1 results in *Lactobacillus* proteins retaining the ability to crystallize. Thus working examples in the

application provide substantive evidence of efficacy and the recited function of crystallizing capability.

The ordinarily skilled person would not require further detailed analysis to reasonably predict locations of insertions which could be effectively made. Claim 1 specifies the regions where insertions may be made and the Examples of the application show those regions to be effective as illustrated, for example, on pages 44 and 45 of the specification. One of ordinary skill in the art will be able to make and use the claimed invention without undue experimentation.

The description describes *Lactobacillus* and its surface layer proteins in detail. *Lactobacillus* and its surface layer protein are described, for example, at page 1, penultimate paragraph to page 2, first paragraph, page 6, third paragraph and over pages 11 to 19. Page 2, first paragraph indicates, for example, that the proteins represent a closely related group of surface layer proteins. Page 6, third paragraph, of the specification lists different *Lactobacilli* and pages 11 to 19 provide, in considerable detail, the *Lactobacilli* to be used and their surface layer proteins. The surface layer proteins to be used in the claimed invention are therefore fully characterized in the specification and will be appreciated by one of ordinary skill in the art, without undue experimentation.

The regions of insertion are also fully characterized in the specification. In particular, for example, support can be found at page 7, lines 3 to 9, page 7, lines 23 and 24 and in pages 44 and 45 of the specification. These passages describe regions recited in claim 1. The regions are therefore described fully and the specification

conveys to the ordinarily skilled person that the applicants were in possession of the claimed invention at the filing date.

(iv) *Presence or absence of working examples*

The specification provides working examples of modified *Lactobacillus* surface layer proteins still able to crystallize. As previously highlighted by Professor Pouwel's declaration in Item 17:

> "*The Examples presented in the application* ***show five different modified*** *Lactobacillus surface layer proteins demonstrating the capacity to form regular two-dimensional crystalline monolayers. Working examples are therefore provided and the invention is reduced to practice.*" [emphasis added]

Furthermore, as noted above, the claimed invention is supported by work of the Examples of the present application. The specification provides a substantial number of examples of the modified *Lactobacillus* S proteins of the claims that retain the ability to form a crystalline monolayer.

(v) *State of the prior art*

The Declaration of Professor Pouwels discusses the Bowie *et al* literature reference cited by the Examiner. In particular, Bowie *et al* is concerned with a wide variety of aspects of protein structure and in particular three-dimensional structure.

As stated in Item 20 of the Declaration of Professor Pouwels:

> "*Bowie et al is concerned with whether insertion of foreign amino acid sequences in a protein will affect the proteins functional properties by changing the three dimensional structure of the protein and change its ability to form a three dimensional crystal of the type used in X ray crystallography.* ***That is not of relevance of the ability of the modified S***

> ***protein to form a two dimensional crystalline monolayer as specified by the claims.*** *Bowie et al does not therefore cast any doubt on the ability of the invention to be put into practice."*[emphasis added]

Thus, Bowie *et al* would not impact the ability of one of ordinary skill to make and use the claimed invention.

The Examiner is requested to appreciate that Bowie *et al* is concerned with making predictions based on sequence analysis. However, the presently claimed invention find support in, for example, the working examples of the specification where modified proteins have been shown to retain the ability to crystallize. There is therefore an important distinction between the claimed subject matter and the teaching of Bowie *et al*.

For instance, Bowie *et al* at page 1306, left hand column, second paragraph states as an aim that:

> "*it should be possible to predict structure from sequence, and subsequently to infer detailed aspects of function from structure*"

However, the present claims do not rely on predictions based solely on the basis of sequence. The claimed subject matter are based on the whole of the present specification, including the example, and the generally advanced level of skill in the art.

Bowie *et al* also compiles the effect of amino acid changes across groups of related proteins to try and provide a model to predict the effect of a given substitution. This modeling has little relevance to the present invention.

The Examiner is further requested to appreciate that Bowie *et al* fail to describe *Lactobacillus* surface layer proteins. Bowie *et al* do not therefore describe or suggest

any specific predictions relating to such proteins. As discussed in the specification itself, these represent a group of closely related proteins for which the teaching of Bowie *et al* is not directly relevant given how the proteins behave similarly.

In addition, the Examiner's attention is again directed to the above Figures 1 to 3 which illustrate similarity of the structure of the different *Lactobacillus* surface layer proteins and which provide evidence that one of ordinary skill in the art will reasonably predict similar behaviors for the proteins. The ordinarily skilled person would have been able to make and use the claimed invention, without undue experimentation. Documents such as Smit *et al* and Boot *et al* discussed above support such a conclusion in showing that regions in S proteins from Lactobacillus would be predicted to behave in an analogous manner. Such documents would have confirmed for the ordinarily skilled person that a region shown to allow insertion and retention of crystallization ability for one S protein would have a similar function for other *Lactobacillus* S proteins.

Thus, nothing in Bowie *et al* provides evidence that an undue burden is placed on the ordinarily skilled person in making and using the claimed invention.

(vi) *Quantity of experimentation necessary*

As set forth above, the claims supported by an enabling disclosure which exemplifies aspects of the claimed products. At most, only a reasonable amount of experimentation would be required to make and use the claimed invention.

The Examiner is again requested to see Item 21 of the Declaration from Professor Pouwels which stated as follows:

> *"The **simple test** taught by the specification and Examples **does not represent undue experimentation** and the skilled person is readily able to both generate modified Lactobacillus S proteins and to assess their ability to form two-dimensional crystalline monolayers as specified by the claims."* [emphasis added]

Thus, the specification illustrates how ability to crystallize can be readily measured and no undue burden is placed on the ordinarily skilled person in determining whether a given protein can crystallize. Undue experimentation would not be required to make and use the claimed invention.

(vii) *Summary*

Claim 1 specifies that the surface layer protein is from a *Lactobacillus* bacterium, as well as specific regions where insertions have been demonstrated to not eliminate the ability of the modified protein to crystallize.

Undue experimentation would not have been required to make and use the claimed invention.

As a final point, claim 40 refers to a slightly larger region in residues 290 to 410. Smit *et al* shows that the region between 290-410 can be deleted without loss of the capacity to form a crystalline structure *in vitro*. Considering that this region can be deleted and that its sole role is the attachment of the crystallization domain of the S-protein (amino acids 1-290) to the bacterial cell wall, it was reasonable to predict that once the experimental work in the application demonstrated insertions within that region were possible and crystallization ability retained, that that would be the case for the entire region *in vitro*.

The claims are supported by an enabling disclosure and withdrawal of the Section 112, first paragraph "enablement", rejection is requested.

To the extent not obviated by the above amendments, the Section 112, first paragraph "written description", rejection of claims 1-8, 33 and 34, is traversed. Reconsideration and withdrawal of the rejection are requested in view of the above and the following comments.

The claims specify both that the protein is from a *Lactobacillus* bacterium and also to refer to the specific regions where insertions have been demonstrated not to eliminate crystallization in the Example of the present application. One of ordinary skill will appreciate that the applicants were in possession of the claimed invention at the time the application was filed.

As discussed above, the Examples of the specification provide representative examples of modified *Lactobacillus* S layer proteins that are able to form crystalline monolayers as specified by the claims. The insertion locations of the claims are described in the specification.

As also discussed above, Lactobacillus surface layer proteins represent a group of closely related proteins which have a great degree of sequence identity and similarity and are structurally similar. The Examiner is requested to see, for example, the following statement made in Item 24 of Professor Pouwel's declaration:

> "*S proteins from Lactobacillus bacteria represent a tightly defined group of proteins. The specific modified S proteins described in the specification do therefore provide a representative and adequate illustration of the invention.*" [emphasis added]

Item 27 of the Declaration of Professor Pouwels also highlighted how the specific modified proteins generated demonstrate that the exemplified products are applicable to *Lactobacillus* S proteins as:

> "*It is reasonable to extrapolate from the results seen in the Examples to S* proteins from Lactobacillus bacteria in general given that such S-proteins have generic properties."

As further stated by Professor Pouwels in Item 27:

> *"All Lactobacillus S-proteins share a number of common characteristics which are not found in non S-layer proteins, the most prominent one being the capacity to form a regular two-dimensional array on the surface of bacteria or in vitro (in the absence of bacteria).* ***These special features allows the extrapolations from the S-protein of L. acidophilus dealt with in the Examples to other S-proteins from Lactobacillus bacteria to be made."*** [emphasis added]

Thus, generalization between *Lactobacillus* surface layer proteins is reasonable and one of ordinary skill in the art will appreciate that the applicants were in possession of the claimed invention at the time the application was filed.

As discussed above Bowie *et al.*, cited by the Examiner, is not believed to be relevant to the claimed products.

Withdrawal of the Section 112, first paragraph "written description", rejection is requested.

The Section 112, second paragraph, rejection of claim 7 will be moot upon entry of the present Amendment. Entry of the present Amendment is requested to at least reduce this issue for appeal.

The claims are submitted to be in condition for allowance and a Notice to that effect is requested along with entry of the present Amendment. The Examiner is requested to contact the undersigned in the event anything further is required in this regard.

Respectfully submitted,

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